

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 6395-61750
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. § 1.5) <b>10/009698</b>
INTERNATIONAL APPLICATION NO. PCT/US00/15828	INTERNATIONAL FILING DATE 8 June 2000	PRIORITY DATE CLAIMED 9 June 1999
TITLE OF INVENTION COMPOSITION AND METHOD FOR DETECTING SYPHILIS USING SYNTHETIC ANTIGENS		
APPLICANT(S) FOR DO/EO/US Victoria Pope, Arnold R. Castro, William E. Morrill		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"><li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. § 371.</li><li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. § 371.</li><li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).</li><li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19<sup>th</sup> month from the earliest claimed priority date.</li><li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. § 371(c)(2))<ol style="list-style-type: none"><li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li><li>b. <input type="checkbox"/> has been transmitted by the International Bureau.</li><li>c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li></ol></li><li>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. § 371(c)(2)).</li><li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))<ol style="list-style-type: none"><li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li><li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li><li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li><li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li></ol></li><li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).</li><li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. § 371(c)(4)).</li><li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. § 371(c)(5)).</li></ol>		
<b>Items 11. to 16. below concern document(s) or information included:</b>		
<ol style="list-style-type: none"><li>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.</li><li>12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§ 3.28 and 3.31 and the Recordal fee of \$40.00 is included.</li><li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li><li>14. <input type="checkbox"/> A substitute specification.</li><li>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</li><li>16. <input checked="" type="checkbox"/> Other items or information:<ol style="list-style-type: none"><li><input checked="" type="checkbox"/> Written Opinion.</li><li><input checked="" type="checkbox"/> Preliminary Examination Report.</li><li><input checked="" type="checkbox"/> International Search Report.</li><li><input type="checkbox"/> Copies of References Cited.</li></ol></li></ol>		

**24197**

DJZ:elb 12/05/01 6395-61750 84153

EXPRESS MAIL LABEL NO. EL874429425US  
DATE OF DEPOSIT: December 5, 2001

U.S. APPLICATION NO (if known, see 37 C.F.R. § 1.5) <b>10/009698</b>		INTERNATIONAL APPLICATION NO PCT/US00/15828		ATTORNEY'S DOCKET NUMBER 6395-61750	
17. <input checked="" type="checkbox"/> The following fees are submitted:  <b>BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):</b>  Neither International Preliminary Examination fee (37 C.F.R. § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .....\$1,040.00  International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....\$890.00  International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO as an International Searching Authority.....\$740.00  International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....\$710.00  International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....\$100.00				CALCULATIONS (PTO USE ONLY)	
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>\$ 890.00</b>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	25 - 20 =	5	x \$18.00	\$ 90.00	
Independent Claims	6 - 3 =	3	x \$84.00	\$ 252.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$280.00	\$	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$ 1,232.00</b>	
<input type="checkbox"/> Reduction of 1/2 for filing by small entity. Small entity status is claimed for this application.				\$	
<b>SUBTOTAL =</b>				<b>\$ 1,232.00</b>	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 Months from the earliest claimed priority date (37 C.F.R. §§ 1.492(f)).				\$	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 0.00</b>	
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). \$40.00 per property.				\$ 40.00	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 1,272.00</b>	
				REFUND →	\$
				CHARGE →	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$1,272.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No. <u>02-4550</u> . A duplicate copy of this sheet is enclosed. d. <input checked="" type="checkbox"/> Please return the enclosed postcard to confirm that the items listed above have been received.					
<b>NOTE:</b> Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:  KLARQUIST SPARKMAN, LLP One World Trade Center, Suite 1600 121 S.W. Salmon Street Portland, OR 97204-2988					
<u>William D. Noonan</u> SIGNATURE William D. Noonan, M.D. NAME  <u>Registration No. 30,878</u> REGISTRATION NUMBER					

cc: Client  
Docketing

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Victoria Pope, Arnold R. Castro, Art Unit:  
William E. Morrill

Application No.

Filed: Herewith

For: COMPOSITION AND METHOD FOR  
DETECTING SYPHILIS USING SYNTHETIC  
ANTIGENS

Examiner:

Date: December 5, 2001

BOX PATENT APPLICATION  
U.S. PATENT AND TRADEMARK OFFICE  
P.O. BOX 2327  
ARLINGTON, VA 22202

**PRELIMINARY AMENDMENT**

In the Specification:

Please add the following language:

**--PRIORITY CLAIM**

This is a § 371 U.S. National Stage of PCT/US00/75666, filed June 8, 2000, which was published in English under PCT Article 21(2), which claims the benefit of U.S. Provisional Application 60/138,192, filed June 9, 1999.--

In the Claims:

Prior to examination of the above-identified § 371 National Stage application, please add the following claims:

22. An antigen composition comprising synthetic cardiolipin, synthetic lecithin, cholesterol and ethanol, wherein the concentration of cardiolipin is between approximately 0.02 and 0.04%, the concentration of lecithin is between approximately 0.11 and 0.16%, and the concentration of cholesterol is approximately 0.9%.

23. An antigen composition comprising between approximately 0.02 and 0.04% tetramyristoyl cardiolipin, between approximately 0.11 and 0.16% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, approximately 0.9% cholesterol, and ethanol to volume.

24. An antigen composition comprising approximately 0.03% tetramyristoyl cardiolipin, between approximately 0.11 and 0.16% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and approximately 0.9% natural cholesterol in absolute ethanol to volume.

25. A method for detecting the presence of *Treponema pallidum* in a human comprising:

- (a) obtaining a biological sample from a human;
- (b) combining the biological sample with a composition comprising between approximately 0.02 and 0.04% tetramyristoyl cardiolipin, between approximately 0.11 and 0.16% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, approximately 0.9% cholesterol, and ethanol to volume; and
- (c) detecting an immunocomplex formed between an antibody in the biological sample and the composition.

### **REMARKS**

Claims 1-21 are pending in the instant application. By this amendment, a claim for priority to the previous PCT and provisional applications has been added. Additionally, claims 22-25 have been added. No new matter is added by this amendment. Support for these claims in the specification as filed can be found as follows:

Claim 22	Page 6, line 27 – Page 7, line 9
Claim 23	Page 11, lines 24-28
Claim 24	Page 11, lines 24-28
Claim25	Page 19, line 11- Page 20, line 5

Consideration of the application as amended is respectfully requested.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

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**Marked-up Version of Amended Claims and Specification  
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)**

*In the Specification:*

**At page one:**

**--PRIORITY CLAIM**

This is a § 371 U.S. National Stage of PCT/US00/75666, filed June 8, 2000, which was published in English under PCT Article 21(2), which claims the benefit of U.S. Provisional Application 60/138,192, filed June 9, 1999.--

*In the Claims:*

22. (new) An antigen composition comprising synthetic cardiolipin, synthetic lecithin, cholesterol and ethanol, wherein the concentration of cardiolipin is between approximately 0.02 and 0.04%, the concentration of lecithin is between approximately 0.11 and 0.16%, and the concentration of cholesterol is approximately 0.9%.

23. (new) An antigen composition comprising between approximately 0.02 and 0.04% tetramyristoyl cardiolipin, between approximately 0.11 and 0.16% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, approximately 0.9% cholesterol, and ethanol to volume.

24. (new) An antigen composition comprising approximately 0.03% tetramyristoyl cardiolipin, between approximately 0.11 and 0.16% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and approximately 0.9% natural cholesterol in absolute ethanol to volume.

25. (new) A method for detecting the presence of *Treponema pallidum* in a human comprising:

(a) obtaining a biological sample from a human;

(c) detecting an immunocomplex formed between an antibody in the biological sample and the composition.

Rec'd PCT/PTO 05 DEC 2001

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**METHOD FOR**  
**DETECTING SYPHILIS USING SYNTHETIC ANTIGENS**

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This invention was made by the Centers for Disease Control and Prevention, an agency of the United States Government.

**Field of the Invention**

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The present invention relates to the fields of microbiology and immunology and more specifically relates to compositions and methods for detecting, diagnosing and monitoring the treatment of syphilis. In particular, the invention pertains to synthetic cardiolipin and lecithin antigen compositions and their use in immunoassays.

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**Background of the Invention**

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Syphilis is a sexually transmitted disease (STD) caused by the bacterium *Treponema pallidum*. Over 100,000 cases of adult syphilis are reported worldwide each year. The disease is also transmitted congenitally, affecting 3000 or more infants annually. Failure to obtain antibiotic treatment in the early stages of the disease allows progression of the disease throughout the body, often resulting in irreversible damage to organs, insanity, blindness, or death. The spread of the human



immunodeficiency virus (HIV) around the world has greatly amplified the severity of syphilis as a health problem because genital ulcers produced during the early stages of syphilis infection facilitate the sexual transmission of HIV.

5           The course of syphilis has been divided into stages; primary, secondary, latent, neurosyphilis and tertiary (late). An infected individual may infect others during the first two stages. Transmission occurs when bacteria are spread from the ulcer of an infected person to the skin or mucous  
10           membranes of the genital area, mouth, or anus of a sexual partner. *T. pallidum* organisms can also pass through broken skin on other parts of the body. In tertiary syphilis and neurosyphilis, the bacterial infection is not contagious, but the invasion of the organism into the organs, tissues, and brain can  
15           have fatal consequences such as serious cardiovascular abnormalities or neurologic disease.

          Vertical or transplacental syphilis infection can occur during the first four years a pregnant woman is infected and not treated. Although adequate treatment of the mother  
20           usually prevents congenital syphilis, approximately 25% of human fetuses that have been exposed to *T. pallidum* infection *in utero* are reported as stillbirth deaths. Some infants with congenital syphilis have symptoms at birth, but most develop symptoms two to three months post partum. These symptoms  
25           include skin sores, rashes, fever, swollen liver and spleen, jaundice, anemia, and various deformities. As infected infants mature, they may develop the symptoms of late-stage syphilis including irreversible damage to bones, teeth, eyes, ears, and brain.

30           The first symptom of primary syphilis is an ulcer, or chancre. The chancre appears within ten days to three months after exposure and is usually found on the part of the body that was exposed to the ulcer of an infected sexual partner,

such as the penis, vulva, vagina, cervix, rectum, tongue, or lip. Because the chancre lasts only a few weeks and may be painless or occur inside the body, it may go unnoticed. The chancre disappears with or without treatment. In persons who are untreated, secondary symptoms will appear approximately nine weeks after the appearance of the primary lesion.

Secondary syphilis is often marked by a skin rash that is characterized by brown sores approximately the size of a penny. Because active bacteria are present in these sores, any physical contact, sexual or non-sexual, with the broken skin of an infected individual may spread the infection at this stage. Other symptoms include mild fever, fatigue, headache, sore throat, patchy hair loss, and swollen lymph glands. These symptoms may be mild and, like the chancre of primary syphilis, will disappear with or without treatment. If untreated, the infected person then enters a period of latency.

Latent syphilis is characterized by the absence of clinical signs or abnormal findings in cerebrospinal fluid (CSF) in conjunction with positive results of serologic tests. Early latent syphilis, which occurs within one year of infection, is potentially transmissible and relapses may occur, while late latent syphilis is associated with immunity to relapse and resistance to re-infection.

During the early stages of syphilis infection, the bacteria may invade the nervous system. If left untreated, neurosyphilis may develop. Progression of the disease to neurosyphilis may take up to twenty years, and some individuals having neurosyphilis fail to develop recognizable symptoms, making diagnosis very difficult. Those who do present symptoms may complain of headache, stiff neck, or fever, which result from an inflammation of the lining of the brain. Seizures and symptoms of stroke such as numbness,

weakness, or visual problems may also afflict neurosyphilis patients.

Although approximately two-thirds of *T. pallidum*-infected individuals who fail to obtain treatment will suffer no further consequences of the disease, approximately one-third of those with untreated latent syphilis develop the complications of late, or tertiary, syphilis. In the tertiary stage of syphilis, the bacteria damage the heart, eyes, brain, nervous system, bones, joints, or almost any other part of the body. The tertiary stage can last for years, or even decades. Late syphilis commonly results in cardiovascular disease, mental illness, blindness, or even death.

Due to the sometimes serious and life threatening effects of syphilis infection, and the risk of transmitting or contracting HIV, specific and early diagnosis of the infection is essential. Syphilis, however, has sometimes been referred to as "the great imitator" because its early symptoms are similar to those of many other diseases. Therefore, a physician usually does not depend solely on a recognition of the signs and symptoms of syphilis, but relies on the results of clinical tests including the microscopic identification of syphilis bacteria and analytical tests for manifestations of syphilis infection in biological samples.

Diagnosis of syphilis by microscopic identification of the bacteria is performed generally as follows. A scraping is taken from the surface of the ulcer or chancre and is examined under a special "dark-field" microscope to detect the organism. Dark-field microscopy requires considerable skill and is prone to misinterpretation.

For these reasons, most cases of syphilis are first diagnosed serologically using non-treponemal assays. Non-treponemal tests detect substances, such as antibodies, that are produced in the presence of a *T. pallidum* infection. The

currently available non-treponemal assays most often used to detect evidence of a syphilis infection are the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) test. The VDRL test employs lipids obtained from naturally-occurring sources, to detect anti-lipoidal antibodies that are generated upon infection by *T. pallidum*. These antibodies are generated against the cardiolipin of the *T. pallidum* organism by the immune system of the individual infected with *T. pallidum* and may be found in the serum or cerebrospinal fluid of the individual.

One disadvantage to the presently available non-treponemal tests is poor specificity. Many medical conditions, including mycoplasma infection, pneumonia, malaria, acute bacterial and viral infections, and autoimmune disease can cause false positive test results in presently available tests for syphilis. For example, intravenous drug use or autoimmune disease causes tissue damage, which results in the release of cardiolipin and the production of anti-cardiolipin antibodies. Detection of these anti-cardiolipin antibodies in a non-treponemal test would therefore produce a false positive result. Successful diagnosis is particularly problematic for the detection of neurosyphilis.

Due to the occurrence of false positive and false negative results when using these existing tests, confirmation using an alternative method of analysis, such as microscopy or a treponemal-based serological test, is normally required. Standard treponemal-based tests include the fluorescent treponemal antibody-absorption (FTA-ABS) test and the FTA-ABS double staining test (FTA-ABS DS). Although treponemal-based assays may be used to confirm a positive test result, these tests are often expensive, complicated, and time consuming, and may require the use of highly sophisticated scientific instrumentation and trained scientific personnel. In

addition, treponemal assays cannot be used as tests to monitor the success of antibiotic therapy because, due to the continued presence of anti-*T. pallidum* antibodies after cure, the tests results remain positive even after eradication of the infection for approximately 85% of successfully treated individuals.

Therefore, a single assay for the sensitive and specific detection of *T. pallidum* infection in a sample for the diagnosis of early stage syphilis or neurosyphilis is needed. Also needed is a simple, inexpensive assay that can be used to monitor the success of syphilis treatment.

### Summary of the Invention

An antigen composition and method for detecting *Treponema pallidum* infection and thereby diagnosing syphilis are provided. The antigen composition contains a combination or mixture of synthetic cardiolipin and synthetic lecithin. The preferred antigen composition also contains a cholesterol. The preferred antigen composition further includes an alcohol. The alcohol solubilizes the lipids in the antigen composition to form a suspension. The antigen composition is useful as a reagent in assays for the detection of antibodies associated with *T. pallidum* infection in a biological sample, particularly a body fluid such as serum or cerebrospinal fluid. Preferably, the antigen composition is an immunoassay reagent for the detection or measurement of antibodies associated with *T. pallidum* infection.

The more preferred antigen composition contains purified, synthetic cardiolipin, synthetic lecithin, natural or non-synthetic cholesterol, and an alcohol. The optimal purity of the synthetic cardiolipin and lecithin in the composition is 99% or greater. The optimal purity of the cholesterol is 98% or greater, or is ash free. Most preferably, the antigen composition contains tetramyristoyl cardiolipin, 1-palmitoyl-2-oleoyl-*sn*-

glycero-3-phosphocholine, cholesterol, and absolute (100%) ethanol. The preferred concentration by volume of synthetic cardiolipin in the composition is between approximately 0.02 - 0.04%, more preferably 0.03%. The preferred concentration by volume of synthetic lecithin in the composition is between approximately 0.11 - 0.16%, more preferably 0.14%. The preferred concentration by volume of cholesterol in the composition is approximately 0.9%, and the remainder of the composition is the alcohol.

The antigen composition provided herein is also useful in general as an *in vitro* research tool for studying syphilis. More particularly, the composition is useful in assays or diagnostic kits to detect the presence of *T. pallidum* infection, which is diagnostic or prognostic for the occurrence or recurrence of syphilis disease.

The preferred method provided herein is an immunoassay for the detection of cardiolipin antibodies in a biological sample such as serum or cerebrospinal fluid. In accordance with the method, the antigen composition described herein is combined with the biological sample for a sufficient amount of time under conditions that facilitate the binding of anti-lipoidal antibodies in the sample to a synthetic cardiolipin-lecithin matrix, to form an antibody-antigen complex. This complex is then detected using methods well known to those skilled in the art such as flocculation or microflocculation tests, or the like.

Accordingly, it is an object of the present invention to provide a method for detecting carriers of *T. pallidum* infection and thus prevent the spread of *T. pallidum* from one host to another.

It is another object of the present invention to provide a sensitive method for the diagnosis of early or latent syphilis or neurosyphilis.

It is yet another object of the present invention to provide a rapid, simple, and inexpensive assay for the accurate detection of *T. pallidum*.

5 It is a further object of the present invention to provide an inexpensively produced antigen composition for the reproducible measurement or detection of *T. pallidum*.

It is another object of the present invention to provide a test for the detection of *T. pallidum* offers advantages in the standardization and stability of the VDRL antigen.

10 These and other objects, features, and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

## 15 Detailed Description

An antigen composition and method for the detection of *Treponema pallidum* are described herein. The antigen composition contains a mixture or combination of synthetic cardiolipin and synthetic lecithin. Cardiolipin is a 1,3-bis(phosphatidyl)glycerol having antigenic properties. 20 Lecithin is a phospholipid. The antigen composition preferably also includes a non-synthetic (natural) cholesterol. An alcohol is also a component of the preferred antigen composition. The alcohol solubilizes the lipids, thereby forming a suspension. 25 The synthetic cardiolipin and lecithin have an optimal purity of 99% or greater. The cholesterol has an optimal purity of 98% or greater, or is ash free. The antigen composition is useful as a reagent in assays for the detection of antibodies associated with *T. pallidum* infection in a biological sample. Preferably, the 30 antigen composition is an immunoassay reagent for the detection or measurement of antibodies generated in a patient infected with *T. pallidum*, which is diagnostic or prognostic for the occurrence or recurrence of syphilis.

The method described herein is an assay for the detection or quantitation of antibodies associated with infection of a patient by *T. pallidum* in a biological sample, particularly a body fluid sample such as serum or cerebrospinal fluid. The method permits detection of circulating antibodies associated with *T. pallidum* infection in order to detect or monitor a *T. pallidum* infection. A preferred method provided herein is an immunoassay. In accordance with the preferred method, the antigen composition is combined with the biological sample for a sufficient amount of time under conditions that facilitate the binding of anti-lipoidal antibodies in the sample to the cardiolipin in the antigen composition, to form antibody-antigen complexes. These antibody-antigen complexes are then detected using methods well known to those skilled in the art such as the VDRL flocculation or microflocculation test, which is read microscopically.

### Definitions

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

The term "antibodies" as used herein includes monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, and humanized antibodies as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

The phrases "specifically binds to" or "specifically immunoreactive with", when referring to an antibody, refers to a binding reaction which is determinative of the presence of the antigen of interest in the presence of a heterogeneous population of peptides, proteins, lipids and other biologics. Thus, under designated immunoassay conditions, the specified antigen or antigens bind preferentially to particular antibodies



and do not bind in a significant amount to other antibodies present in the sample. Specific binding under such conditions requires an antigen that is selected for its specificity for a particular antibody. A variety of immunoassay formats may be used to select antigens specifically immunoreactive with a particular antibody. For example, solid-phase ELISA immunoassays are routinely used to select an antigen specifically immunoreactive with an antibody. *See*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

The term "antigen" refers to an entity or fragment thereof which can induce an immune response in a mammal. The term includes immunogens and regions responsible for antigenicity or antigenic determinants. The term "antigen composition" as used herein refers to a composition containing synthetic cardiolipin and synthetic lecithin. "Antigenic determinant," as used herein, refers to a region of an antigen that is recognized by an antibody.

As used herein, the terms "detecting" or "detection" refers to qualitatively or quantitatively determining the presence of the biomolecule under investigation.

By "isolated" is meant a biological molecule free from at least some of the components with which it naturally occurs.

#### Antigen Compositions

The composition provided herein contains a combination, suspension or physical mixture of one or more synthetic cardiolipins and lecithins. A preferred composition contains synthetic cardiolipin, synthetic lecithin, and synthetic or non-synthetic (naturally occurring) cholesterol. The more

preferred composition contains synthetic cardiolipin, synthetic lecithin, natural cholesterol, and an alcohol.

The preferred concentration of synthetic cardiolipin in the composition is between approximately 0.02 - 0.04% by volume, more preferably 0.03% by volume. The preferred concentration of synthetic lecithin in the composition is between approximately 0.11 and 0.16% by volume, more preferably 14% by volume. The preferred concentration of natural cholesterol in the composition is approximately 0.9% by volume, and the remainder of the composition is alcohol, preferably ethanol, most preferably absolute (100%) ethanol.

The synthetic cardiolipin may be synthesized from semi-synthetic lipid precursors originating from plant sources. In a most preferred embodiment, the cardiolipin is tetramyristoyl cardiolipin, which is commercially available from sources such as Avanti Polar Lipids (Alabaster, AL).

The synthetic lecithin may be derived from soybeans or egg. In a preferred embodiment, the lecithin is a 16:0, 18:1 lecithin also described as 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine or 3-*sn*-phosphatidylcholine (*sn* means stereospecifically numbered), which is also available commercially from sources such as Avanti Polar Lipids (Alabaster, AL).

In a most preferred embodiment, the composition is a suspension containing approximately 0.03% tetramyristoyl cardiolipin, 0.11-0.16% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and 0.9% natural cholesterol in absolute ethanol. The cholesterol is also available from commercial sources such as Avanti Polar Lipids (Alabaster, AL). The alcohol can be purchased from chemical suppliers such as Sigma Chemical Company (St. Louis, MO).

When combined together with the alcohol, the cardiolipin, lecithin, and cholesterol form a lipid matrix or

micelle. As described in more detail below, antibodies associated with the presence of a human *T. pallidum* infection, referred to herein as anti-cardiolipin antibodies, bind to these lipid micelles and form antibody-antigen complexes. Therefore, the detection or measurement of these antibody-antigen complexes can be used to diagnose a *T. pallidum* infection. Although not wishing to be bound by the following hypothesis, it is believed that the anti-cardiolipin matrix antibodies bind to the synthetic antigen composition described herein with greater specificity and higher avidity than they bind to naturally occurring cardiolipin and lecithin. The synthetic antigen composition thereby provides a more efficient, more sensitive, and more specific means to detect antibodies associated with a syphilis infection.

It will be understood by those skilled in the art that one or more components of the antigen composition can be labelled with a detectable label to facilitate the direct measurement or detection of antibody-antigen complex formation. Various types of labels and methods of conjugating the labels to the antigen composition are well known to those skilled in the art.

Also, the antigen composition may be employed as a laboratory research tool to generate, isolate or purify anti-cardiolipin antibodies, and the antibodies can be used to study syphilis in general. Therefore, the antigen composition is useful for purposes such as *in vivo* and *in vitro* diagnostics and laboratory research.

#### Preparation of the VDRL Antigen

The VDRL antigen can be prepared, for example, by preparing an ethanolic solution of tetramyristoyl cardiolipin at a concentration by volume ranging from 0.02 to 0.04%, more preferably 0.03%. An ethanolic solution of synthetic lecithin

having a concentration by volume of approximately 0.11 to 0.16%, more preferably 0.14%, and an ethanolic solution of natural cholesterol of 0.9% are added to the cardiolipin solution. The components are added in the following sequence: cardiolipin, lecithin, cholesterol, and ethanol to volume. The antigen is solubilized and stored at room temperature overnight before testing.

#### Detection of Anti-Cardiolipin Antibodies

The method provided herein includes diagnostic and prognostic methods to detect and quantify antibodies capable of binding to the antigen composition described above. These methods permit detection of circulating antibodies to the cardiolipin-lecithin matrix in order to indicate the presence of *T. pallidum* infection and thereby diagnose infection or monitor the progress of an antibiotic in treating a *T. pallidum* infection.

There are many techniques known in the art for detecting or measuring antibody-antigen complexes, also referred to herein as immunocomplexes. Classical methods involve reacting a sample containing the antibody with a known excess amount of the antigen specific for the antibody, separating bound from free antigen, and determining the amount of bound antigen or free antigen. If free antigen is measured, the amount of bound antigen can be calculated by subtracting the amount of free antigen from the known starting amount. Often the antigen is directly or indirectly labeled with a reporter group or detectable label to aid in the determination of the amount of antibody-antigen complex as described herein. The reporter group or "label" is commonly a fluorescent or radioactive group or an enzyme. The label is then detected using methods well known to those skilled in the art such as spectrophotometry, scintillation counting, or flow cytometry.

Alternatively, the antigen can be conjugated to a solid phase bead or particle that is filtered, centrifuged, or otherwise removed from the mixture, such as by magnetically removing a metallic or magnetized particle. Attaching the antigen to solid phase beads, such as latex beads, provides a new more sensitive and rapid slide agglutination test.

In a preferred embodiment, the antigen is attached to the beads through the cardiolipin molecule. One method of attaching the antigen to the beads is to modify the cardiolipin molecule so that it can be covalently bonded to the beads. For example, an amine group can be attached to the terminal methyl groups of the fatty acid chains of the cardiolipin. Cardiolipin modified in this manner can be attached to carboxylated or aminealated latex beads.

A preferred immunoassay for the detection of anti-cardiolipin antibodies in a sample is performed as follows. A sample is collected or obtained using methods well known to those skilled in the art. The sample containing the anti-cardiolipin antibodies to be detected is obtained from a biological source. The sample is preferably obtained from a biological fluid, such as, but not limited to, whole blood, blood serum, blood plasma, saliva, cerebrospinal fluid, and the like. Optimal diagnostic results are obtained when the sample is serum or spinal fluid. The sample may be filtered or otherwise manipulated prior to immunoassay to optimize the immunoassay results.

The sample is then incubated with the antigen composition described herein to form an antibody-antigen immunocomplex. The antibody-antigen complex is then detected using methods well known to those skilled in the art. The term "detecting" or "detected" as used herein means using known techniques for detection of biologic molecules such as immunochemical or histological methods. Such methods

include immunological techniques employing monoclonal or polyclonal antibodies to the lipids, such as enzyme linked immunosorbant assays (ELISA), sandwich assays, flow cytometric assays, radioimmunoassays, or other types of assays involving antibodies known to those skilled in the art.

In a preferred embodiment of the method, anti-cardiolipin antibodies in a sample are detected by employing the synthetic antigen composition described herein in a flocculation assay. Examples of known flocculation assays include the unheated serum reagin test (USR), the rapid plasma reagin 10 mm circle card test (RPR), the toluidine red unheated serum test (TRUST) and the VDRL slide assay. These assays are all flocculation tests. It will be understood by those skilled in the art that the anti-cardiolipin antibodies are also detectable using the antigen composition described herein in agglutination tests, which further employ carrier particles. In a more preferred embodiment of the method, the synthetic antigen composition is used in a VDRL slide assay as described briefly below and in more particularly in *Venereal Disease Research Laboratory (VDRL) Slide Test*, Kennedy, E.J. Jr. and Creighton, E.T., 157-78 (1998) in *A Manual of Tests for Syphilis*, 9<sup>th</sup> ed., Larsen, S.A., Pope, V., Johnson, R.E. and Kennedy, E.J. Jr. (Eds.), American Public Health Association, Washington, D.C., which is incorporated by reference herein.

The VDRL slide assay is performed as follows. VDRL-buffered saline containing formaldehyde,  $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{NaCl}$  and distilled water, is placed in a vessel. The antigen composition is added slowly to the saline at a constant rate while rotating the vessel and the mixture is then agitated to thoroughly combine the contents and form a suspension. The sample, such as serum, is placed into a ring of a paraffin or ceramic-ringed slide and one drop of the antigen composition suspension is added. The slide is rotated to mix the sample and

antigen composition and is then read microscopically. The presence of clumps, clumping or roughness indicates antibody-antigen formation. Serial dilutions of the antigen suspension can be used for a qualitative measurement of antibody in the sample. Quantitative determinations can be made by performing the assay with standard concentrations of antibody and comparing the results of the sample with the results generated by the standards.

It is to be understood that the assay methods are contemplated to include the use of synthetic antigen compositions as described above and synthetic derivatives of the antigen compositions described herein provided that the derivatives retain antigenic activity or display an equivalent antigenic activity and have specificity for anti-cardiolipin antibodies.

#### Kit for Detecting the Presence of *T. pallidum*

A kit for diagnosing or otherwise evaluating a syphilis infection by detecting the presence or quantity of anti-cardiolipin antibodies is also provided. The kit can be in any configuration well known to those of ordinary skill in the art and is useful for performing one or more of the methods described herein for the detection of anti-cardiolipin-lecithin matrix antibodies in biological samples or for the detection or monitoring of *T. pallidum* infection in a patient or carrier. The kits are convenient in that they supply many if not all of the essential reagents for conducting an assay for the detection of syphilis antibodies in a biological sample. The reagents may be pre-measured and contained in a stable form in vessels or on a solid phase in or on which the assay may be performed, thereby minimizing the number of manipulations carried out by the individual conducting the assay. In addition, the assay may be performed simultaneously with a standard that is included with

the kit, such as a predetermined amount of antibody, so that the results of the test can be validated or measured.

The kit preferably contains the antigen composition described herein, which can be used for the detection of cardiolipin antibodies associated with *T. pallidum* infection. The kit also preferably contains cholesterol and can additionally contain the appropriate reagents that aid in detecting antibody-antigen complexes. The kit may additionally contain equipment for safely obtaining the sample, a vessel for containing the reagents, a buffer for diluting the sample or reagents, and circle cards such as the 10 mm slides or 18 mm circle cards used in VDRL, RPR and TRUST assays.

The assay kit includes but is not limited to reagents to be employed in the following techniques; flocculation tests such as USR, RPR and TRUST; agglutination assays; and sandwich or ELISA assays. Materials used in conjunction with these techniques include, but are not limited to, microtiter plates, antibody-coated strips or dipsticks for rapid monitoring of biological fluids. For each kit, the range, sensitivity, precision, reliability, specificity and reproducibility of the assay are established. Standardization may be achieved using reference control sera and titering the sera to endpoint or a panel of sera may be utilized.

In a more preferred embodiment, the assay kit uses VDRL slide techniques and provides instructions and the antigen composition described above. The kit is useful for the measurement of *T. pallidum* infection, and more specifically, for the measurement of antibodies directed toward cardiolipin in biological fluids of humans exhibiting symptoms of syphilis or those at risk for syphilis infection.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the



contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

### Example 1

#### *Preparation of a Synthetic Cardiolipin and Lecithin Composition*

Tetramyristoyl cardiolipin, purified by silica gel chromatography to approximately 99% purity, was obtained in powder form from Avanti Polar Lipids (Alabaster, AL). The final concentration of sodium salt was tested for purity by thin layer chromatography and high-pressure liquid chromatography. The sample was stored at -20° C. The tetramyristoyl cardiolipin was originally synthesized from semi-synthetic lipid precursors that originated from a plant source.

Lecithin (1-palmitoyl-2-oleoyl-*sn*-glycero-phosphocholine) powder, purified by silica gel chromatography to a purity of approximately 99%, was also obtained from Avanti Polar Lipids. The lecithin was originally isolated from soybeans.

A 1.2% solution of cholesterol (Avanti Polar Lipids) in absolute ethanol was prepared and filtered with alcohol-rinsed filter paper #560. The cholesterol was originally derived from wool grease and purified by re-crystallization, and the crystals were stored at -20° C.

An antigen composition was prepared by combining the synthetic cardiolipin with the synthetic lecithin, the cholesterol solution and the ethanol, in that order. The final

concentration of synthetic cardiolipin was 0.02-0.03% by volume. The final concentration of synthetic lecithin was 0.11-0.16% by volume. The final concentration of cholesterol was 0.9% by volume and the remainder of the antigen composition was the ethanol.

### Example 2

#### *Comparative Analysis of Synthetic VDRL Slide Assay versus Conventional VDRL Slide Assay*

The sensitivity of the VDRL slide assay using the synthetic cardiolipin and lecithin composition described in Example 1 was compared with the sensitivity of the conventional VDRL slide assay as described in *A Manual of Tests for Syphilis*, 9<sup>th</sup> ed., 159-77, Larsen, S.A., Pope, V., Johnson, R. E. and Kennedy, E.J. Jr. (Eds.), American Public Health Association, Washington, D.C. Briefly, 0.4 ml VDRL-buffered saline (formaldehyde, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, NaCl and distilled water) was added to the bottom of a round 30 ml glass-stoppered bottle with a flat inner-bottom surface or a 25 ml glass-stoppered Erlenmeyer flask. Subsequently, 0.5 ml of the antigen composition suspension was added directly to the saline at a rate of 6 seconds/0.5 ml of antigen suspension while rotating the bottle continuously. Rotation continued for ten seconds until 4.1 ml of buffered saline was added. The bottle was tightly capped and shaken from bottom to top approximately thirty times in ten seconds. The antigen suspension was used within eight hours.

The qualitative tests were performed by placing 50 µl of serum into one ring of a paraffin or ceramic-ringed slide using a safety pipetting device. The antigen suspension was gently re-suspended and one free-falling drop (17 µl) was added. The slide was placed on a mechanical rotator for four

minutes at  $180 \pm 2$  rpm. The slide was immediately removed and read microscopically using 10X oculars and a 10X objective. Results were reported as follows: reactive - medium or large clumps, weakly or minimally reactive - small clumps, nonreactive - no clumping or a very slight roughness. Quantitative tests were similarly performed with serial two-fold dilutions of the serum.

The results, set forth below in Table 1, indicated that the test using the synthetic antigen composition was more sensitive than the test using the standard VDRL antigen, which was made using natural cardiolipin and lecithin.

**Table 1**  
**Comparison of Sensitivities of Natural VDRL versus Synthetic VDRL Assays**

Test	Primary	Sensitivity	
		Secondary	Latent
Natural VDRL	80%	100%	85%
Synthetic VDRL	84%	100%	88%

The sensitivities of the VDRL slide assay using the synthetic cardiolipin and lecithin antigen composition described in Example 1 and the conventional VDRL slide assay were also compared against the conventional RPR slide assay. As shown in Tables 2 and 3 below, the assay using the synthetic VDRL antigen composition was more reactive with samples that tested positive using the RPR test than the assay using the non-synthetic VDRL antigen.

**Table 2**  
**RPR versus Synthetic VDRL**

	RPR	<u>Synthetic VDRL</u>	
		Reactive	Nonreactive
5	<u>Reactive*</u>	41	13
	<u>Nonreactive</u>	1	5
	*All RPR reactives were minimally reactive		

**Table 3**  
**RPR versus Natural VDRL**

	RPR	<u>Natural VDRL</u>	
		Reactive	Nonreactive
10	<u>Reactive*</u>	13	41
15	<u>Nonreactive</u>	0	6
	*All RPR reactives were minimally reactive		

### **Example 3**

#### **Comparative Analysis of Synthetic VDRL antigen and Natural VDRL antigen (Qualitative Test)**

Samples from 100 frozen banked sera, reactive by the nontreponemal (RPR) test, were used to compare the CDC synthetic VDRL antigen and a reference VDRL antigen (Natural VDRL antigen). The serum samples were heat inactivated for 30 minutes at 56°C. Fifty microliters of each serum sample was placed into a corresponding paraffin or ceramic-ringed slide. A drop (17µL) of each of the antigens was placed in the corresponding rings of the slide. The slides were placed in a mechanical rotator and rotated for 4 minutes at 180 rpm and then read microscopically. The degree of flocculation of the two antigens was observed and recorded.

As reported in Table 4 (undocumented) all of the sera (100%), reactive by RPR, were reactive with the CDC synthetic VDRL antigen while only 88% were reactive with the natural VDRL antigen.

Additionally, the synthetic VDRL antigen and the natural VDRL antigen were compared in the same test using 100 samples from documented syphilis cases. The results of this test are also shown in Table 4 (documented). All of the results from these tests were confirmed by the SERODIA *Treponema pallidum* particle agglutination test (TP-PA) (Fujirebio America, Inc., Fairfield, NJ).

**Table 4**

Syphilis Category	Number of Serum Samples	Number of serum samples reactive with		
		Synthetic VDRL antigen	Natural VDRL antigen	TP-PA
Undocumented	100	100	88	99
Documented				
Untreated Primary	9	9	9	8
Secondary	20	20	20	20
Latent	6	5	5	6
Treated Primary	15	12	11	13
Secondary	30	30	30	30
Latent	20	18	17	19
Total	200	194	180	195

#### Example 4

##### *Comparative Analysis of Synthetic VDRL antigen and Natural VDRL antigen (Quantitative Test)*

Samples from 100 frozen banked sera, reactive by the nontreponemal (RPR) test, were used to compare the CDC

synthetic VDRL antigen and a reference VDRL antigen (Natural VDRL antigen). The serum samples were diluted two-fold in a test tube with 0.9% saline. Fifty microliters of each of the tube dilutions was transferred to the corresponding rings of a ceramic or paraffin-ringed slide. A drop (17 $\mu$ L) of each of the antigens was placed in the corresponding rings of the slide. The slides were placed in a mechanical rotator and rotated for 4 minutes at 180 rpm. The endpoint titer of each of the serum dilutions was read microscopically. One doubling dilution difference was defined as an endpoint either (R) from one antigen or (N) for the other antigen.

As seen in Table 5 (undocumented), this test showed that 85% of the frozen banked sera reactive in the RPR test had end-point titers of one half or one dilution greater with CDC synthetic VDRL antigen than with natural VDRL antigen. In 15% of the cases, the end-point titer obtained with the CDC synthetic VDRL antigen was equal to that obtained with the natural VDRL antigen. In none of the samples tested was the end-point titer greater with the natural antigen than with the CDC synthetic antigen.

This test was repeated using 100 samples from documented syphilis cases. As seen in Table 5 (documented), 84% of the sera from documented syphilis cases had end-point titers of one half or one dilution greater with CDC synthetic VDRL antigen than with natural VDRL antigen. In 7% of the cases, the end-point titer obtained with the CDC synthetic VDRL antigen was equal to that obtained with the natural VDRL antigen, while in 3% of the cases the end-point titer of the natural VDRL antigen was one half or one dilution greater than that of the CDC synthetic VDRL antigen. The results of these tests were confirmed by TP-PA test.

**Table 5**

Syphilis Category	Number of Specimens	CDC Synthetic VDRL antigen higher	Natural VDRL antigen higher	CDC synthetic and Natural VDRL antigens equal endpoints
Undocumented	100	85	0	0
Documented				
Untreated    Primary	9	3	2	4
Secondary	20	20	0	0
Latent	6	4	0	1
Treated        Primary	15	10	0	2
Secondary	30	30	0	0
Latent	20	17	1	0
Total	200	169	3	7

**Example 5**

5        *Comparative Analysis of Synthetic VDRL antigen and Natural  
VDRL antigen (Qualitative Test) in patients having diseases  
other than Syphilis*

10        Samples from 100 patients having diseases other  
than syphilis were qualitatively tested using the procedure of  
Example 3. These tests were confirmed by the TP-PA test and  
the FTA-ABS test. The results of these tests are reported in  
Table 3 which shows that all of the samples were nonreactive  
with both the CDC synthetic VDRL antigen and the natural  
VDRL antigen. Four of the samples were reactive in the TP-  
15        PA test, but nonreactive in the FTA-ABS test.

**Table 6**

Category of Sample	Number of Specimens	Number of serum samples reactive and nonreactive							
		CDC Synthetic VDRL antigen		Becton Dickinson VDRL antigen		TP-PA		FTA-ABS	
		R	N	R	N	R	N	R	N
Rheumatic Fever	27	0	27	0	27	4	23	0	27
Coronary Arterial Disease	9	0	9	0	9	0	9	ND	
Hypertension	6	0	6	0	6	0	6	ND	
Diabetes	4	0	4	0	4	0	4	ND	
Parkinson's	2	0	2	0	2	0	2	ND	
Obesity	2	0	2	0	2	0	2	ND	
Angina	2	0	2	0	2	0	2	ND	
Miscellaneous Category	48	0	48	0	48	0	48	ND	
Total Number	100	0	100	0	100	4	96		

R= reactive; N=nonreactive

5

**Example 6**

*Comparative Analysis of Synthetic VDRL antigen and Natural VDRL antigen (Qualitative Test) in biological false positive samples*

10 Samples were obtained from 50 individuals that were originally classified as biological false positives (BFP). These individuals tested nontreponemal test reactive and treponemal test nonreactive. These samples were tested using the procedure of Example 3. The results of this test is shown in Table 7. Four of the serum samples that were originally

15 misclassified as BFP were found reactive with the CDC



synthetic VDRL antigen, the TP-PA, and the FTA-ABS tests. Three of these four samples were also reactive with the natural VDRL antigen.

5

**Table 7**

Reactivity of Sample	TP-PA	CDC synthetic VDRL antigen	Natural VDRL antigen
Reactive	4	28	27
Nonreactive	46	22	23

R= reactive; N=nonreactive

**Example 7**

10

*Comparative Analysis of Synthetic VDRL antigen and Natural VDRL antigen (Qualitative Test) in unknown samples*

15

495 samples with no patient identifiers were tested with CDC synthetic VDRL antigen and natural VDRL antigen using the procedure in Example 3 above. Reactive specimens were confirmed with the TP-PA test, the ELISA test for syphilis IgG antibody, or the FTA-ABS test. Thirty-eight of the samples were reactive in one of the treponemal tests and 457 were nonreactive. As can be seen in Table 8, all of the samples that were treponemal reactive were reactive with the CDC synthetic VDRL antigen and 36 were reactive with the natural VDRL antigen. Of the 457 serum samples that were treponemal nonreactive, 452 were nonreactive with the CDC synthetic VDRL antigen and 450 were nonreactive with the natural VDRL antigen.

20

25

**Table 8**

	Number of Tests	CDC synthetic VDRL antigen		Natural VDRL antigen	
		R	N	R	N
Reactive	38	38	0	36	2
Nonreactive	457	5	452	7	450

R= reactive; N=nonreactive

We claim:

1. An antigen composition comprising synthetic cardiolipin and synthetic lecithin.

5 2. The antigen composition of Claim 1, further comprising cholesterol.

3. The composition of Claim 2, wherein the concentration of cholesterol is approximately 0.9%

4. The composition of Claim 2, further comprising an alcohol.

10 5. The composition of Claim 1, wherein the concentration of cardiolipin is between approximately 0.02 and 0.04%.

6. The composition of Claim 5, wherein the concentration of cardiolipin is approximately 0.03%.

15 7. The composition of Claim 1, wherein the concentration of lecithin is between approximately 0.11 and 0.16%.

8. The composition of Claim 7, wherein the concentration of lecithin is approximately 0.14%.

20 9. The composition of Claim 1, wherein the cardiolipin is tetramyristoyl cardiolipin.

10. The composition of Claim 1, wherein the lecithin is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

25 11. The composition of Claim 4, wherein the alcohol is ethanol.

12. A method for detecting the presence of *Treponema pallidum* in a human comprising combining a biological sample from the human with a composition comprising synthetic cardiolipin and synthetic lecithin and detecting an immunocomplex formed between an antibody in the biological sample and the composition.

13. The method of Claim 12, wherein the composition further comprises cholesterol and an alcohol.

14. The method of Claim 13, wherein the concentration of cholesterol in the composition is approximately 0.9%.

15. The method of Claim 12, wherein the alcohol is ethanol.

16. The method of Claim 12, wherein the concentration of cardiolipin in the composition is between approximately 0.01 and 0.05%.

17. The method of Claim 12, wherein the concentration of lecithin in the composition is between approximately 0.11 and 0.16%.

18. The method of Claim 12, wherein the cardiolipin in the composition is tetramyristoyl cardiolipin.

19. The method of Claim 12, wherein the lecithin in the composition is 1-palmitoyl-2-oleoyl-*s n*-glycero-3-phosphocholine.

20. The method of Claim 12, wherein the detection of an immunocomplex is a positive diagnosis of syphilis.

21. The method of Claim 12, wherein the immunocomplex is detected using a flocculation or agglutination test.

**COMBINED DECLARATION AND POWER OF ATTORNEY  
FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled COMPOSITION AND METHOD FOR DETECTING SYPHILIS USING SYNTHETIC ANTIGENS, the specification of which

- ☐ is attached hereto.
- ☐ was filed on \_\_\_\_\_ as United States Application No. \_\_\_\_\_.
- ☒ was described and claimed in PCT International Application No. PCT/US00/15828, filed on June 8, 2000.
- ☐ and was amended on \_\_\_\_\_ (if applicable).
- ☐ with amendments through \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of an PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)	Priority Claimed	
	<input type="checkbox"/> Yes	<input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

<u>60/138,192</u>	<u>June 9, 1999</u>
(Application No.)	(Filing Date)

✓ J16

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US00/15828	June 8, 2000	Pending
(Application No.)	(Filing Date)	(Status: patented, pending, abandoned)

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from \_\_\_\_\_ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

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**24197**

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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